Mode of Action of Colicin A

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Colicin A acting on sensitive cells of Escherichia coli inhibits a number of energy-requiring cellular functions. It appears to act similarly to colicins E1 and K.

Different colicins exert their lethal action by affecting different cellular mechanisms. Thus colicins E1 and K halt all macromolecular synthesis soon after adsorption; colicin E2 induces deoxyribonucleic acid degradation, and colicin E3 affects primarily the protein-synthesizing machinery (7).

A study of the mode of action of colicin A was prompted by the finding of mutants which in one step acquired insensitivity to the three colicins of group E and to colicins K and A (6, 9, 10). Colicin A has been characterized as different from the other known colicins because it selects specific resistant mutants (4) and because of its serological specificity (1). The present results indicate that colicin A affects macromolecular synthesis in a way similar to that of colicins E1 and K.

Colicin A was prepared from Escherichia coli 06:H16, 23, by the same procedure used for E1 and K (6). Incorporation of 14C-uracil into acid-insoluble material was measured to test the effect of colicin on nucleic acid synthesis. As shown in Fig. 1, uracil incorporation is halted very soon after addition of the colicin. No effect on uracil incorporation was observed after addition of the colicin to a mutant resistant to colicin A.

Colicin A, like E1 and K (2), blocks certain permease activities. Figure 2 shows the results of tests on accumulation of several substrates. The uptake of 14C-isoleucine was blocked (Fig. 2A) and already accumulated isoleucine was released (Fig. 2B). Similar effects were observed on the accumulation of thio-methyl-β-D-galactoside (not shown in Fig. 2). These activities of colicin A, like those of colicins E1 or K, are exerted also in the presence of chloramphenicol. The accumulation of α-methyl glucoside, which is mediated by a phospho-enol-pyruvate-dependent phosphorylation system (5, 11) and is insensitive to colicins E1 and K, is also unaffected by colicin A (Fig. 2C). Like colicins E1 and K (2), colicin A potentiates the inhibition of α-methyl glucoside accumulation by sodium fluoride (NaF).

Addition of colicin A to a colicin-sensitive, actively motile culture of E. coli K-12 caused a complete arrest of motility within 2 or 3 min, as seen under the phase microscope. In contrast to colicin E2, colicin A did not induce vegetative replication of phage λ in a lysogenic sensitive strain (8).

All these observations are consistent with the hypothesis that colicin A affects macromolecular syntheses and the adenosine triphosphate (ATP)-

![Graph](http://jb.asm.org/Downloadedfromhttp://jb.asm.org/)
Fig. 2. Effects of colicin A on substrate accumulation. (2A) Cells of strain C600 were grown in Tris-glycerol supplemented with the required growth factors, chilled, centrifuged, and resuspended in fresh medium without supplements, and chloramphenicol (50 μg/ml) was added. After 30 min at room temperature, the culture was split into two portions; one received colicin A (survival 0.1%), and the other was served as a control. 14C-Isoleucine (10⁻⁸ μ, 0.025 μc/ml) was added to both suspensions 5 min later. Samples taken at different times were collected on Millipore membranes, washed with ice-cold buffer, dried, and counted. (2B) Chase of 14C-isoleucine. A bacterial suspension like the control sample in Fig. 2A received 14C-isoleucine (2 × 10⁻⁸ μ, 0.05 μc/ml). Five minutes later the culture was distributed in two flasks, one containing cold isoleucine (final concentration 4 × 10⁻⁴ μ), the other containing colicin A. Samples were treated as in Figure 2A. (2C) Effect of colicin A and NaF on accumulation of α-methylglucoside (αMG). E. coli K-12, strain 3300, was grown in Tris-glucose medium, and the cells were washed as described for Fig. 2A. After addition of chloramphenicol, the suspension was equilibrated at room temperature and divided in 4 flasks: flask 1 had colicin A, flask 2 had NaF (final concentration 50 μm), flask 3 contained both NaF and colicin A, and flask 4 was a control. After 5 min, 14C-α-MG was added to each flask (2.5 × 10⁻⁴ μ, 0.025 μc/ml). Ten minutes later cold αMG (final concentration 2 × 10⁻⁹ μ) was added to the flask with colicin A. Samples taken at different times were treated as described for Fig. 2A.

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